Enantiomeric Determination of Methamphetamine, Amphetamine, Ephedrine, and Pseudoephedrine using Chiral Supercritical Fluid Chromatography with Mass Spectrometric Detection

Li Li, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[li.li2@usdoj.gov]

ABSTRACT: Supercritical fluid chromatography-single quadrupole mass spectrometry (SFC-SQD) was utilized for the enantiomeric determination of methamphetamine, amphetamine, ephedrine, and pseudoephedrine. The effects of varying stationary phases, alcohol modifiers, and amine additives were assessed. The optimal separation for methamphetamine was achieved by a Trefoil AMY1 (150 x 2.1 mm, 2.5 µm) column using a supercritical CO_2 mobile phase containing EtOH as the co-solvent and 1% cyclohexylamine as the amine additive. The method was successfully applied for determination of the chiral composition of illicit methamphetamine, even for samples with skewed ratios of enantiomers as low as 0.1% d- or 3% l-.

KEYWORDS: Methamphetamine, Amphetamine, Ephedrine, Pseudoephedrine, Chiral Separation, Supercritical Fluid Chromatography, Mass Spectrometry, Forensic Chemistry.

Introduction

The d- and l- enantiomers of methamphetamine have dramatically different pharmacological activity levels (1,2). Despite these differences, both enantiomers are Schedule II in the U.S., due to their widespread abuse[1]. In addition, samples containing 80% or more d-methamphetamine HCl are subject to higher sentences [3]. Therefore, the enantiomeric composition of methamphetamine is important from legal and medical perspectives. In addition, the chirality of methamphetamine can help indicate the possible synthetic route and precursor used in its synthesis. d-Methamphetamine is most commonly produced either from l-ephedrine or d-pseudoephedrine, while racemic d,l-methamphetamine is most commonly produced from 1-phenyl-2-propanone (P2P; also known as phenylacetone and by several other, more obscure names and acronyms). Methamphetamine with a skewed ratio of d- and l- is usually synthesized from P2P with subsequent, incomplete resolution using d-tartaric acid or a similar agent [4]. Thus, the enantiomeric composition of methamphetamine is also important for forensic and intelligence derivation purposes.

Since the enactment of the Combat Methamphetamine Epidemic Act of 2005 [5], which implemented purchasing limits on l-ephedrine and d-pseudoephedrine, the chiral composition of methamphetamine seized in the U.S. has shifted

[1] Pharmaceuticals containing low concentrations of l-methamphetamine, which are used as bronchodilators, are not controlled.

dramatically, from the d- enantiomer and the d,l-racemate to samples with skewed ratios of the d- and l- isomers. This shift confirms the current use of P2P and resolution efforts by illicit processors.

Chromatographic enantioseparations remain the most popular techniques for chiral analyses, and can be accomplished using HPLC, GC, CE, and supercritical fluid chromatography (SFC). Direct HPLC using a chiral stationary phase (CSP) is the most common technique for the separation of enantiomers [6-17]. However, this method consumes large quantities of HPLC-grade organic solvents (which are expensive), and can display significant peak broadening as well as longer equilibration and analysis times. In addition, multiple pilot columns with different CSPs are normally needed to determine the optimal column and conditions, another time consuming expense.

Indirect HPLC with chiral mobile phase additives has also been utilized for chiral analyses of phenethylamines; however, the results displayed low efficiency in enantioselectivity [18,19]. Similarly, CE with chiral mobile phase additives is effective, efficient, and rapid [20-24]; however, this method suffers from low reproducibility in migration times, and relative migration times versus internal standards are usually required.

Another common approach involves derivatization with a chiral reagent (to form diasteromers) with subsequent analyses with either HPLC or (more commonly) GC [25-34]. Though such analyses have a better enantioselectivity, efficiency, and reproducibility, the derivatization process is time consuming, more expensive, and technique sensitive, and the optical impurity in the chiral reagents often obscures or masks the detection of the low level isomer in a heavily skewed-ratio sample (chiral derivatizing reagents are typically about 98% enantiomerically pure).

SFC employs mobile phases comprised of super-critical CO_2 mixed with organic modifiers (e.g., alcohols) and additives (e.g., acids or bases); this results in higher diffusivity and lower viscosity versus an HPLC mobile phase. As a result, SFC offers high throughput capacity, much lower solvent consumption, and a "greener," less expensive technology. Recently, with improved instrumentation and wider availability of chiral columns with small particle sizes, SFC has become a viable alternative chromatographic technique for enantiomeric analyses [35-44].

The use of SFC on chiral amylose (AMY) or cellulose (CEL) columns for enantomeric determination of methamphetamine, amphet-amine, ephedrine, and pseudoephedrine is presented herein. Chiral analyses of seized methamphetamine samples with skewed enantiomer ratios are also presented. The effects of varying the stationary phases, co-solvents, and additives are discussed.

Experimental

Chemicals

All phenethylamine standards were obtained from this laboratory's reference materials collection. Ammonium hydroxide solution, ammonium acetate, trifluoroacetic acid (TFA), and cyclohexylamine (CHA) were analytical grade and obtained from Sigma (St. Louis, MO). HPLC grade solvents, including isopropyl alcohol (IPA), ethanol (EtOH), methanol (MeOH), and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). Carbon dioxide (CO_2, beverage grade) was from Air Gas (Chantilly, VA).

Columns

Trefoil AMY1 (tris-(3,5-dimethylphenyl-carbamate) 2.1 x 50 mm, 2.5 µm), Trefoil CEL1 (tris-(3,5-dimethylphenylcarbamate)-cellulose, 2.1 x 50 mm, 2.5 µm), Trefoil CEL2 (tris-(3-chloro-4-

methylphenylcarbamate)-cellulose, 2.1 x 50 mm, 2.5 µm), and Trefoil AMY1 (2.1 x 150 mm, 2.5 µm) columns were provided by the Waters Corporation (Milford, MA).

Instrumentation

All experiments were performed on a Waters Acquity Ultra Performance Convergence Chromatography (UPC^2) System. The system was equipped with a binary solvent manager, an autosampler with a partial loop volume injection system, a 2-position column oven compatible with 150 mm length columns, and was interfaced with a PDA detector and an SQD with an ESI source. A 515 pump system (an isocratic solvent manager) was used as a make-up pump, and was positioned before the mass detector. The main flow stream was split by a flow-splitter assembly before the SQD. Empower 3® software was used for system control and data acquisition.

Screening experiments were performed at 40^0C with a flow rate of 1.2 mL/min. The system back pressure was set at ABPR = 2000 psi, except as noted. The gradient elution was varied to compensate for different modifiers, as specified for each experiment. The injection volume was 2 µL.

The compounds were detected in positive ESI mode with the following parameters: The ion source temperature was 150^0C; nitrogen was used as the desolvation gas at a flow rate of 600 L/hr and at 400^0C; the capillary voltage was 3 kV. The makeup flow was 0.6 mL/min with MeOH. In this study, Single Ion Recording (SIR) was utilized for better sensitivity and selectivity (mass spectral parameters are listed in Table 1).

Standard and Sample Preparation

Stock solutions of each standard were prepared at 1 mg/mL in MeOH. Working solutions of individual standards or their mixtures were prepared by diluting their stock solutions with IPA. For samples, a 1 mg/mL stock solution in MeOH was prepared. A working sample solution at 200 µg/mL was prepared by diluting its stock solution with IPA.

Table 1. Mass Spectral Parameters.

Compound	$[M+H]^+$	Cone Voltage (kV)
Amphetamine	135.8	20
Methamphetamine	150.2	20
Ephedrine	166.2	15
Pseudoephedrine	166.2	15

Results and Discussion

Screening of Chiral Columns with Different Co-Solvents and Additives

Initial screening of stationary phases was attempted using methamphetamine on 50 mm Trefoil "pilot" columns (CEL1, CEL2, and AMY1). Based on Waters' strategy for Trefoil chiral method development, the following four step process was implemented: 1. AMY1-EtOH/IPA/ACH (33/33/33)-20 mM AmAc (B1); 2. CEL1-MeOH/IPA (50/50)-0.2% TFA (B2); 3. CEL2-EtOH/ACN (50/50)-0.2% TFA (B3); 4. AMY1-EtOH/IPA (50/50)-0.2% TFA (B4). The instrument was equilibrated at 3% B for 0.5 min, then linear gradient to 60% B over 1.5 min, then hold at 60% B for 5 min.

There was no noticeable enantiomeric separation of any of the phenethylamines with any of the screening columns and conditions. Distorted peaks were observed, possibly due to the acidic character of the large percentage of CO_2 in the mobile phase. According to a study by Ye *et al.*, addition of an amine raises the pH of the mobile

phase, thereby deprotonating the analytes and improving their peak shapes and resolution [36]. Thus, three organic co-solvents (MeOH, EtOH, or IPA) doped with NH_4OH or CHA were investigated. Only the AMY1 column using an alcohol and CHA demonstrated any chiral separations (and those were slight). Multiple experiments were conducted (not presented herein); based on the results, a longer AMY1 column (150 x 3.5 mm, 2.1 µm) with various alcohols as a co-solvent and CHA as an additive was selected for method optimization.

Screening of Co-Solvents with an AMY1 Chiral Column and using CHA as an Additive

As amphetamine, ephedrine, and pseudoephedrine can also be present in seized methamphetamine samples, it is useful to resolve all four compounds as well as their enantiomers in a single run, if possible. A standard mixture containing 100 µg/mL of all four phenethylamines was prepared in IPA. The effects of MeOH, EtOH, and IPA were separately assessed, each using CHA as an additive. The results are illustrated in Figures 1, 2, and 3. All four phenethylamines were separated using any of the alcohols; however, none of the alcohols resolved all four pairs of enantiomers in a single run. IPA and EtOH are both adequate co-solvents for enantiomeric separation of amphetamine; indeed, d- and l-amphetamine were well resolved using IPA, with resolution greater than 2. The four ephedrine and pseudoephedrine diastereomers were partially resolved by each of the alcohols, with MeOH giving the highest discrimination. Based on these results, MeOH/EtOH (50/50) and 0.3% CHA was utilized, and baseline resolved all four ephedrine/pseudoephedrine diastereomers (Figure 4). The amphetamine enantiomers were also baseline resolved; however, the methamphetamine enantiomers were not quite fully resolved, with the best separation being achieved using EtOH as a co-solvent, with a resolution of 0.9.

Effects of CHA Concentration and Optimization of the Chiral Separation for Methamphetamine

In order to fully resolve the separation of d- and l-methamphetamine, the CHA concentration was raised from 0.3% to 0.5% to 1%, using EtOH as the co-solvent. The flow rate was also increased to 2.5 mL/min for higher efficiency. As illustrated in Figure 5, EtOH with 1.0% CHA separated d- and l- methamphetamine with a resolution of 1.2. Under these conditions, the amphetamine enantiomers were also baseline resolved; however, the separations of ephedrine and pseudoephedrine deteriorated at 1% CHA. Higher percentages of CHA were not investigated due to ion suppression effects.

Detection Limits and Linearity of Methamphetamine Analyses

Using the optimized conditions (SIR mode), the detection limit was 0.2 µg/mL and the linearity range was 0.5 - 200 µg/mL. The detection limits for the minor isomer in a methamphetamine sample with a skewed ratio of enantiomers was also studied. Trace d-methamphetamine could be detected at 0.1%; however, trace l-methamphetamine could only be detected at 3%, due to peak tailing and limited resolution (Figure 6). In contrast to these results, the detection levels for both d- and l- methamphetamine are 0.1% using this laboratory's current CE method [37].

Chiral Determination of Methamphetamine in Seized Samples

Six illicit methamphetamine samples were analyzed using EtOH doped with 1% CHA. The chromatograms are illustrated in Figure 7. The results are compared against the analyses performed using our current CE method in Table 2. To summarize, SFC offers faster and more consistent analyses, but is not well suited for detecting trace levels of l-methamphetamine due to its lower efficiency and resolution.

Table 2. Comparison of the chiral analysis of 6 illicit methamphetamine samples.

Sample	By SFC	By CE
1	d-	0.5% l-
2	d-	0.7% l-
3	d-	0.4% l-
4	l-	l-
5	d-	d-
6	35.4% l-	35.6% l-

Conclusions

Using MeOH/EtOH (50/50) and 0.3% CHA, all four phenethylamines were resolved, and all but the methamphetamine enantiomers were baseline resolved (methamphetamine 0.9). Using EtOH and 1% CHA, the methamphetamine enantiomers were fully resolved (1.2). Both experiments were carried out in less than 7 minutes.

Using SQD in positive ESI and SIR mode, the detection limits were as low as 0.2 µg/mL for methamphetamine. The method also gave a good linearity range for methamphetamine (0.5 to 200 µg/mL). However, the method can only detect l-methamphetamine at 3% or greater. Despite this limitation, the method is an excellent alternative to other GC, HPLC, and CE methods for rapid chiral analyses, with good sensitivity, selectivity, and reliability.

Acknowledgments

The author thanks Mr. Kenneth Blakeslee from Waters Corporation for his assistance and support, and the Waters Corporation for the use of the Acquity UPC^2 system and columns.

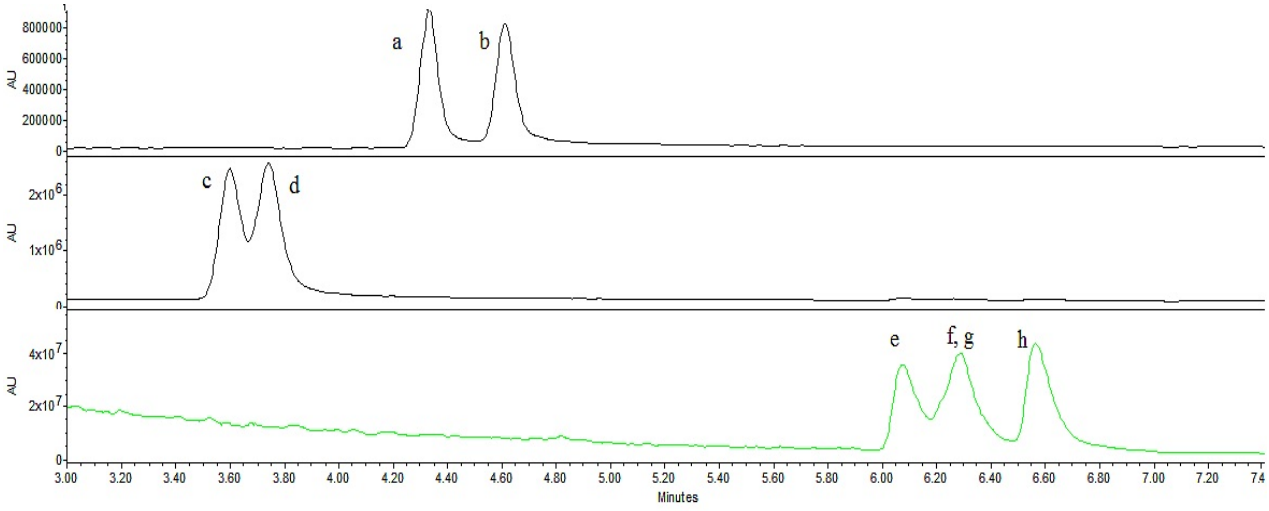

Figure 1. SFC chromatograms of co-solvent screening. Co-solvent: IPA with 0.6% CHA; and gradient: 8% to 15% in 4 min; 15% to 40% at 6 min. Flow rate: 1.5 mL/min. a, d-methamphetamine; b, l-methamphetamine; c, d-amphetamine; d, l-amphetamine; e, d-pseudoephedrine; f, l-pseudoephedrine; g, d-ephedrine; h, l-ephedrine. [Note: Peaks were identified via analyses of individual enantiomers.]

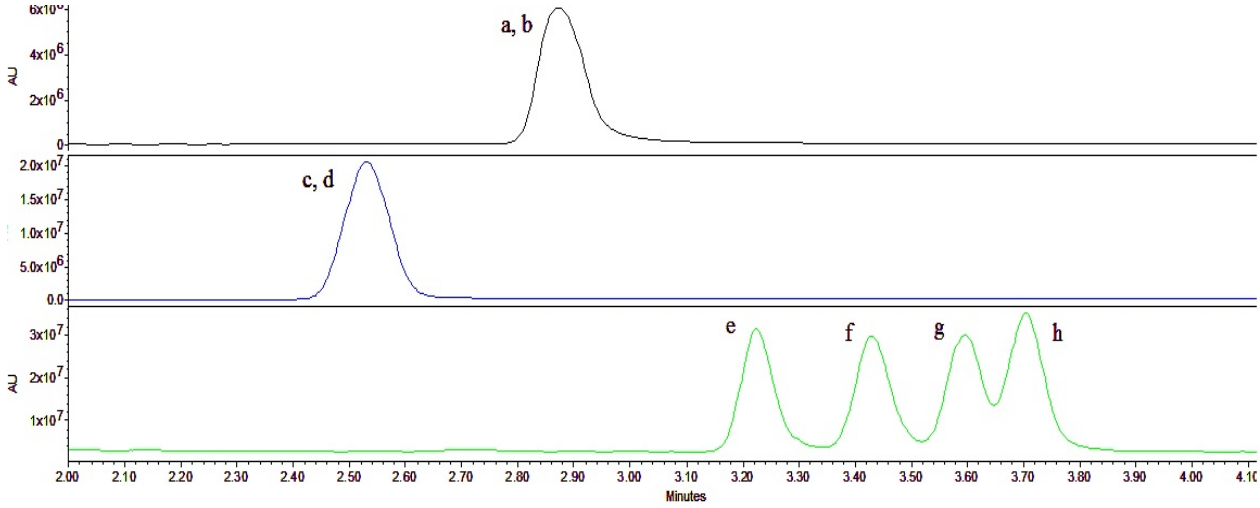

Figure 2. SFC chromatograms of co-solvent screening. Co-solvent: MeOH with 0.3% CHA; and gradient: 8% to 15% in 6 min. Flow rate: 1.2 mL/min.

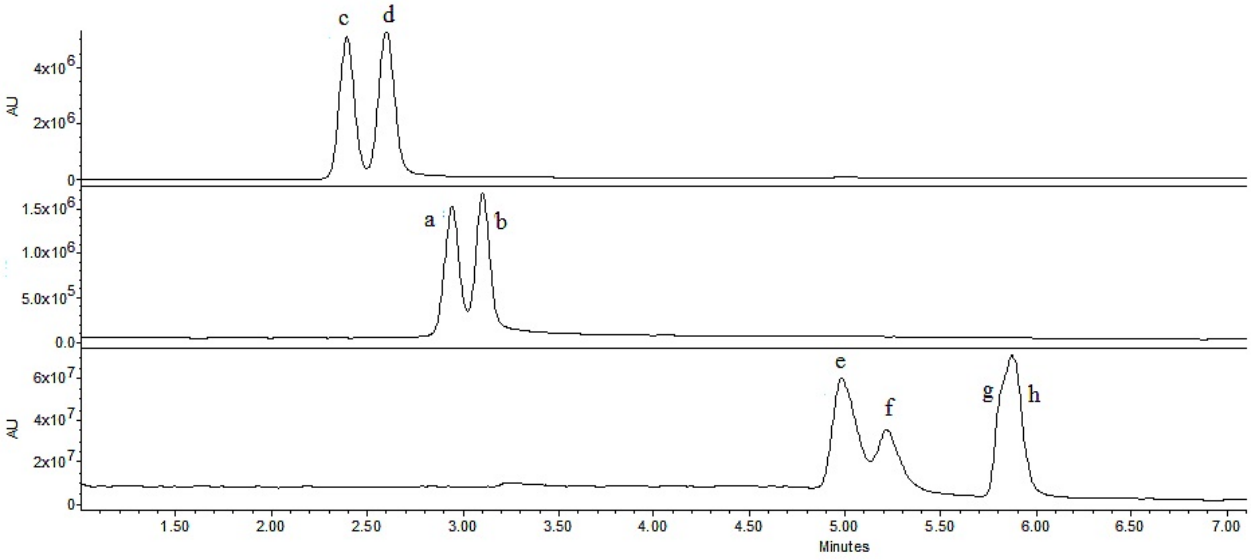

Figure 3. SFC chromatograms of co-solvent screening. Co-solvent: EtOH with 0.5% CHA; and gradient: 8% for 4 min; 8% to 30% at 9 min. Flow rate: 2.0 mL/min.

[Figures Continued, Next Two Pages]

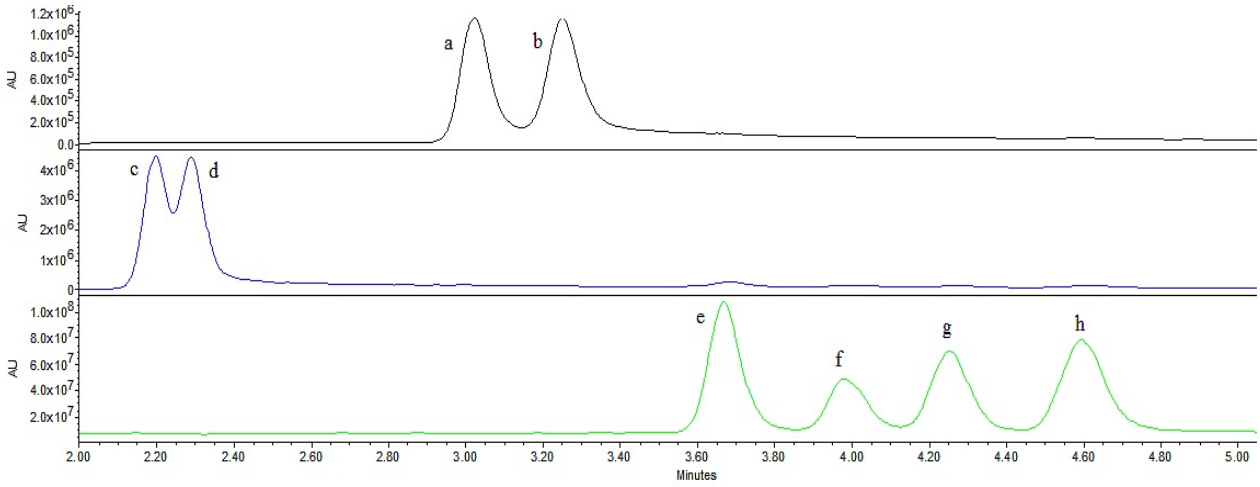

Figure 4. SFC chromatograms of co-solvent screening. Co-solvent: MeOH/EtOH (50/50) with 1.0% CHA; and gradient: 5-7.5% in 6 min. Flow rate: 2.5 mL/min.

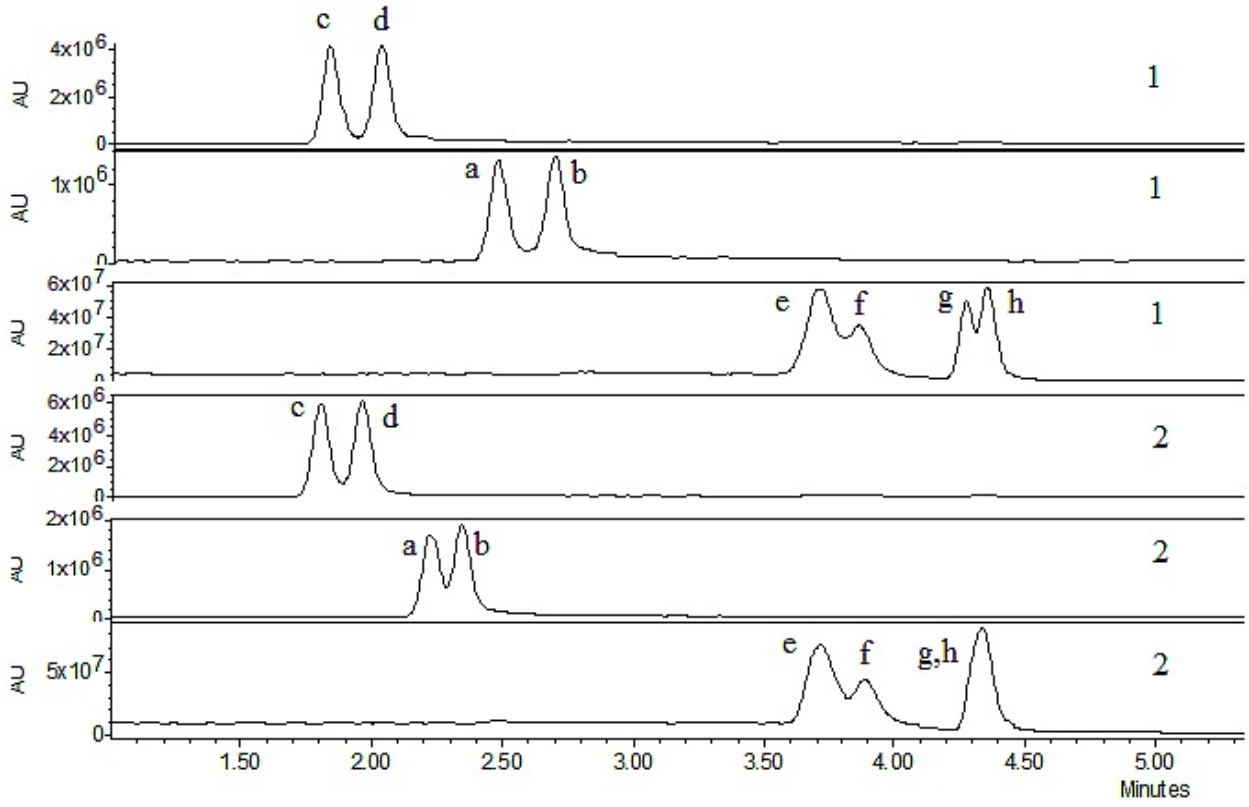

Figure 5. Study of CHA concentration on methamphetamine chiral separation. Co-solvent: EtOH and CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min. 1: 1.0 % CHA; 2: 0.5% CHA.

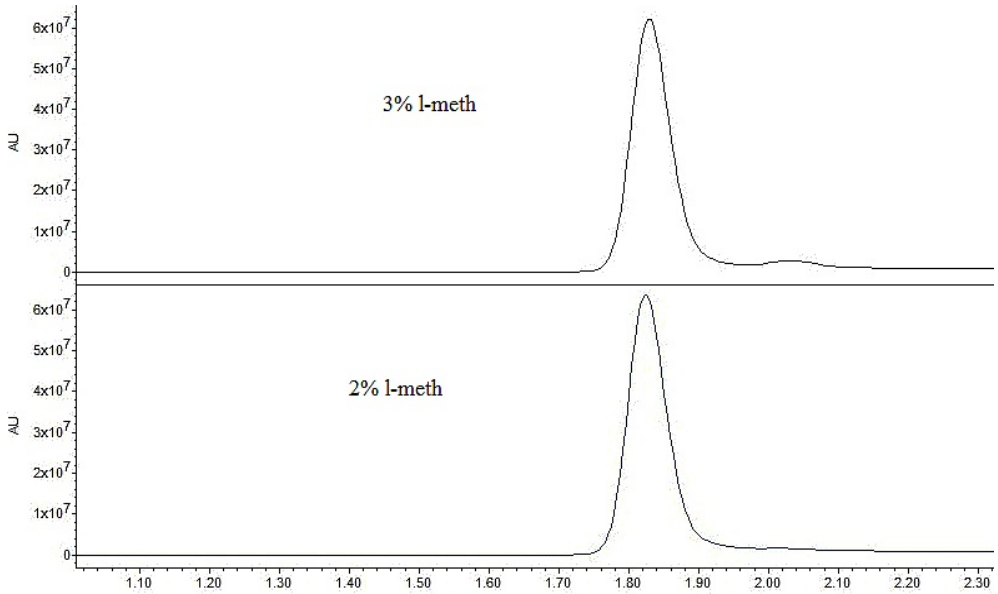

Figure 6. Detection limit of l-methamphetamine (peak at ~2.03 minutes) with a skewed ratio of enantiomers. Co-solvent: EtOH and 1.0% CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min.

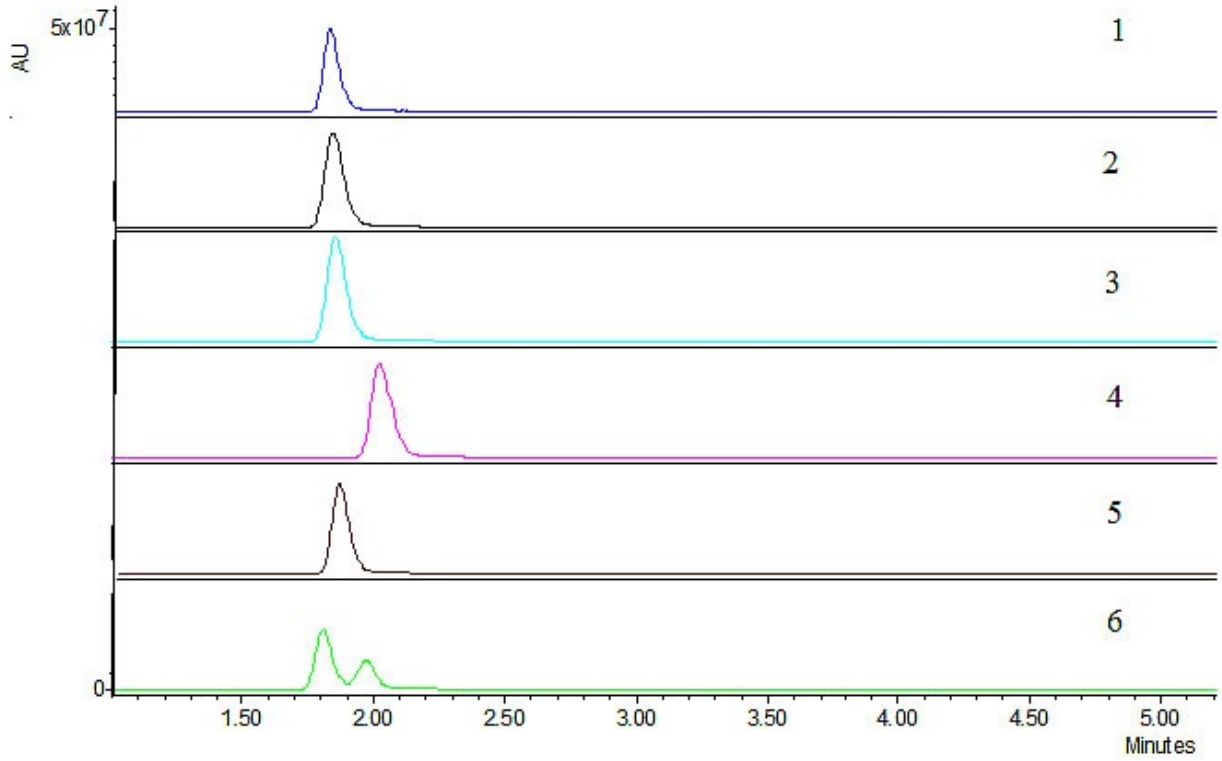

Figure 7. Chiral analysis of methamphetamine samples. Co-solvent: EtOH with 1.0% CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min.

References

1. Maier NM, Franco P, Lindner PW. Separation of enantiomers: Needs, challenges, and perspectives. Journal of Chromatography A 2001;906:3-33.

2. Jirovsky D, Lemr K, Sevcik J. Smysl B, Stránsky Z. Methamphetamine - Properties and analytical methods of enantiomer determination. Forensic Science International 1998;96:61-70.

3. Federal Sentencing Guidelines Manual. 2015, Chapter 2 D. See: http://www.ussc.gov/guidelines-manual/2015/2015-individual-chapters-and-guidelines-html [Date of Last Access December 1, 2015.]

4. Morelato M, Beavis A, Tahtouh M, Ribaux O, Kirkbride KP, Roux C. The use of methylamphetamine chemical profiling in an intelligence-led perspective and the observation of inhomogeneity within seizures. Forensic Science International 2015;246:55-64.

5. Title VII of the PATRIOT Improvement and Reauthorization Act of 2005 (Public Law 109-177).

6. Tachibana K, Ohnishi A. Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases. Journal of Chromatography A 2001;906:127-154.

7. Zhang T, Nguyen D, Franco P. Reversed-phase screening strategies for liquid chromatography on polysaccharide-derived chiral stationary phases. Journal of Chromatography A 2010;1217:1048-1055.

8. Franco P, Zhang T. Common approaches for efficient method development with immobilized polysaccharide-derived chiral stationary phases. Journal of Chromatography B; Analytical Technologies in the Biomedical and Life Sciences 2008;875:48-56.

9. Armstrong DW, Rundeltt KL, Reid III GL. Enantioresolution of amphetamine, methamphetamine, and Deprenyl (Selegiline) by LC, GC, and CE. Current Separations 1996;15(2):57-61.

10. Rizzi AM, Hirz R, Cladrowa-Runge S, Jonsson H. Enantiomeric separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a β-cyclodextrin-chiral stationary phase. Chromatographia 1994;39:131-137.

11. Pihlainen K, Kostiainen R. Effect of the eluent on enantiomer separation of controlled drugs by liquid chromatography-ultraviolet absorbance detection-electrospray ionization mass spectrometry using vancomycin and native β-cyclodextrin chiral stationary phases. Journal of Chromatography A 2004;1033:91-99.

12. Lemr K, Jirovsky D, Sevcik J. Effect of some parameters on enantiomer separation of ephedrine, methamphetamine and Selegiline using HPLC with β-cyclodextrin stationary phase. Journal of Liquid Chromatography and Related Technologies 1996;19 (19):3173-3191.

13. Makinoa Y, Uranob Y, Naganob T. Impurity profiling of ephedrines in methamphetamine by high-performance liquid chromatography. Journal of Chromatography A 2002;947:151-154.

14. Lekskulchai V. Isomeric separation of methamphetamine by HPLC chiral column. Journal of the Medical Association of Thailand 2001;84(11):1604-1613.

15. Phinney KW, Sander LC. Liquid chromatographic method for the determination of enantiomeric composition of amphetamine and methamphetamine in hair samples. Journal of Analytical and Bioanalytical Chemistry 2004;378(1):144-149.

16. Katagi M, Nishioka H, Nakajima K, Tsuchihashi H, Fujima H, Wada H, Nakamura K, Makino K. Direct high-performance liquid chromatographic and high performance liquid chromatographic-thermospray-mass spectrometric determination of enantiomers of methamphetamine and its main metabolites amphetamine and p-hydroxymethamphetamine in human urine. Journal of Chromatography B; Analytical Technologies in the Biomedical and Life Sciences 1996;676:35-43.

17. Newmeyer MN, Concheiro M, Huestis MA. Rapid quantitative chiral amphetamine liquid chromatography-tandem mass spectrometry: Method in plasma and oral fluid with a cost-effective chiral derivatizing reagent. Journal of Chromatography A 2014;1358:68-74.

18. Herráez-Hernández R, Campins-Falcó P. Chiral separation of ephedrines by liquid chromatography using β-cyclodextrins. Analytica Chimica Acta 2001;434:315-324.

19. Taschwer M, Seidl Y, Mohr S, Schmid MG. Chiral separation of cathinone and amphetamine derivatives by HPLC/UV using sulfated ß-cyclodextrin as chiral mobile phase additive. Chirality 2014;26:411-418.

20. Li L, Lurie IS. Regioisomeric and enantiomeric analyses of 24 designer cathinones and phenethylamines using ultra high performance liquid chromatography and capillary electrophoresis with added cyclodextrins. Forensic Science International 2015;254:148-157.

21. Liau AS, Liu JT, Lin LC, Chiu YC, Shu YR, Tsai CC, Lin CH. Optimization of a simple method for the chiral separation of methamphetamine and related compounds in clandestine tablets and urine samples by β-cyclodextrin modified capillary electrophoresis: A complementary method to GC-MS. Forensic Science International 2003;134:17-24.

22. Iwata YT, Inoue H, Kuwayama K, Kanamori T, Tsujikawa K, Miyaguchi H, Kishi T. Forensic application of chiral separation of amphetamine-type stimulants to impurity analysis of seized methamphetamine by capillary electrophoresis. Forensic Science International 2006;161:92-96.

23. Lurie IS, Bozenko Jr. JS, Li L, Miller EE, and Greenfield SJ. Chiral separation of methamphetamine and related compounds

using capillary electrophoresis with dynamically coated capillaries. Microgram Journal 2011;8(1):21-28.

24. Lurie IS. Separation selectivity in chiral and achiral capillary electrophoresis with mixed cyclodextrins. Journal of Chromatography A 1997;792:297-307.

25. Gal J. Resolution of the enantiomers of ephedrine, norephedrine and pseudoephedrine by high-performance liquid chromatography. Journal of Chromatography 1984;307:220-223.

26. Inagaki S, Hirashima H, Taniguchi S, Higashi T, Min JZ, Ruri Kikura-Hanajiri R, Godab Y, Toyo'oka T. Rapid enantiomeric separation and simultaneous determination of phenethylamines by ultra high performance liquid chromatography with fluorescence and mass spectrometric detection: Application to the analysis of illicit drugs distributed in the Japanese market and biological samples. Drug Testing and Analysis 2012;4:1001-1008.

27. Rasmussen LB, Olsen KH, Johansen SS. Chiral separation and quantitation of R/S-amphetamine, R/S-methamphetamine, R/S-MDA, R/S-MDMA, and R/S-MDEA in whole blood by GC-EI-MS. Journal of Chromatography B; Analytical Technologies in the Biomedical and Life Sciences 2006;842:136-141.

28. Drake SJ, Morrison C, Smith F. Simultaneous chiral separation of methamphetamine and common precursors using gas chromatography/mass spectrometry. Chirality 2011;23:593-601.

29. Mohr S, Weiss JA, Spreitz J, Schmid MG. Chiral separation of new cathinone- and amphetamine-related designer drugs by gas chromatography-mass spectrometry using trifluoroacetyl-l-prolyl chloride as chiral derivatization reagent. Journal of Chromatography A 2012;1269:352-359.

30. Coutts RT, Dawe R, Jones GR, Liu SF, Midha KK. Analysis of perfluoroacyl derivatives of ephedrine, pseudoephedrine and analogues by gas chromatography and mass spectrometry I. Trifluoroacetyl derivatives. Journal of Chromatography A 1980;190:53-65.

31. Sardela VF, Sardela PDO, Pereira HMG, Aquino Neto FR. Consequences of the formation of 3,4-dimethyl-5-phenyl-1,3-oxazolidine on the analysis of ephedrines in urine by gas chromatography and a new method for confirmation as N-trifluoroacetyl-O-t-butyldimethylsilyl ether derivatives. Journal of Chromatography A 2011;1218:1266-1272.

32. Wang SM, Lewis RJ, Canfield D, Li TL, Chen CY, Ray H. Liu RH. Enantiomeric determination of ephedrines and norephedrines by chiral derivatization gas chromatography-mass spectrometry approaches. Journal of Chromatography B; Analytical Technologies in the Biomedical and Life Sciences 2005;825:88-95.

33. LeBelle MJ, Savard C, Dawson BA, Black DB, Batyal LK, Zrcek F, By AW. Chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine and methcathinone by gas chromatography and nuclear magnetic

resonance. Forensic Science International 1995;71:215-223.

34. Husk Jr. T. Phenethylamine isomer determination via α-methoxy-α-trifluoro-methyl-phenylacetic acid (MTPA) by gas chromatography. DEA Laboratory Note, May 16, 2014.

35. Klerck KD, Mangelings D, Heyden YV. Review of supercritical fluid chromatography for the enantioseparation of pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis 2012;69:77- 92.

36. Ye YK, Lynam KG, Stringham RW. Effect of amine mobile phase additives on chiral subcritical fluid chromatography using polysaccharide stationary phases. Journal of Chromatography A 2004;1041:211-217.

37. Welch CJ, Biba M, Gouher JR, Kath G, Augustine P, Houek P. Solving multicomponent chiral separation challenges using a new SFC tandem column screening tool. Chirality 2007;19:184-189.

38. Klerck KD, Mangelings D, Clicq D, Boever FD, Heyden YV. Combined use of isopropylamine and trifluoroacetic acid in methanol-containing mobile phases for chiral supercritical fluid chromatography. Journal of Chromatography A 2012;1234:72-79.

39. Klerck KD, Heyden YV, Mangelings D. Exploratory data analysis as a tool for similarity assessment and clustering of chiral polysaccharide-based systems used to separate pharmaceuticals in super-critical fluid chromatography. Journal of Chromatography A 2014;1326:110-124.

40. Biba M, Regaladoa EL, Wu N, and Welch CJ. Effect of particle size on the speed and resolution of chiral separations using supercritical fluid chromatography. Journal of Chromatography A 2014;1363:250-256.

41. Khater S, West C. Development and validation of a supercritical fluid chromatography method for the direct determination of enantiomeric purity of provitamin B5 in cosmetic formulations with mass spectrometric detection Journal of Pharmaceutical and Biomedical Analysis 2015;102:321-325.

42. Stringham RW, Blackwell JA. "Entropically driven" chiral separations in supercritical fluid chromatography. Confirmation of isoelution temperature and reversal of elution order. Analytical Chemistry 1996;68:2179-2185.

43. Plotka JM, Biziuk M, Morrison C, Namies´nik J. Pharmaceutical and forensic drug applications of chiral super-critical fluid chromatography. Trends in Analytical Chemistry 2014;56:74-89.

44. Subbarao L, McCauley J, Sidhu H, Chen R. Enantiometric resolution, identification, and quantitation of chiral illicit drugs using SFC APCI MS. www.waters.com/poster [Date of Last Access July 1, 2015.]

* * * * *